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Report of the Research Briefing Panel on the Biology of Parasitism

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Research Briefing Panel on the Biology of Parasitism

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Parasites are organisms that require another organism, a "host," for survival and that usually do some harm to the host. The field of parasitology has been limited to those organisms that belong to the animal kingdom, although bacteria, viruses, and fungi are also parasites. Of concern here are the single-celled protozoans, such as cause malaria and leishmania; multicellular worms or helminths, such as schistosomes and filaria; and arthropods, which include insects. Many of the arthropods also are vectors that transmit the protozoan and helminthic parasites.

There are two major reasons for the current intense interest in the study of parasites. First, such studies will have a large impact on our understanding of basic biologic processes such as those involved in cell growth and differentiation. Many of the human parasites have multiple hosts and may have several developmental stages in each host. Each stage involves regulated transitions from one form to another. Further, parasites have evolved very specialized adaptations that enable them to infect the host and then evade the host's defense mechanisms while they survive and replicate, utilizing for these purposes the host's metabolic processes. The mechanisms

by which parasites adapt to their environment are diverse and ingenious, and are only beginning to be understood.

Second, application of the knowledge obtained in these basic investigations promises to have a major impact on world health. Of the six diseases singled out for research emphasis by the Special Program for Research and Training in Tropical Diseases, sponsored by the World Bank, the United Nations Development Program, and the World Health Organization, five are caused by parasites: malaria, schistosomiasis, filariasis, trypanosomiasis, and leishmaniasis. These and other parasites affect more than a billion people worldwide. Approximately 300 million suffer from malaria, 200 million have schistosomiasis, and 300 million have filariasis. Another 40 million have onchocerciasis, which causes "river blindness."

Parasite diseases also affect the United States. Giardia, a protozoan parasite, is one of the most common causes of epidemic infectious diarrhea in this country. In addition, we must consider the immigration of a large number of people with parasitic diseases, the vast outflow of American tourists and business representatives overseas,

and the potential exposure of military personnel in countries where parasitic infections are common.

The potential rewards of studying parasites are enormous, both in terms of learning about basic biologic processes and in combating disease. Because of their complexity, animal parasites are difficult to study. However, modern biologic concepts and advances in techniques are now enabling researchers to address such exciting and long-standing questions about parasite-host interactions as the following: (1) How are these unicellular and multicellular organisms capable of establishing infections? (2) What genes regulate the transformation of parasites through their complex life cycle? (3) What are the mechanisms of disease syndromes that result from parasitic infections? (4) What is the array of possible host protective responses and can these be enhanced? (5) What mechanisms do parasites use to evade the host's immune and other protective defenses? (6) Are vaccines feasible? (7) What biochemical pathways do parasites have that differ from the host and can these be used as targets for new drugs? (8) What is the basis for the ability of certain insects to transmit parasites or to become resistant to insecticides, and can these properties be altered?

Some of the technical advances that have been crucial to the study of parasites are the production of monoclonal antibodies, the isolation of specific genes, and the culturing and growth of some of these organisms in the laboratory. This paper describes some basic biologic questions that are being studied in parasites by several different scientific disciplines, and then suggests potential future applications of such information to prevention and treatment of parasitic diseases.

BASIC RESEARCH ON PARASITES

MOLECULAR BIOLOGY

Antigenic Variation

African trypanosomiasis is characterized by cyclic parasitemia, that is, by the appearance

in the blood stream every 7 to 10 days of waves of organisms. The surface of the protozoan's membrane is covered by a single type of glycoprotein, which is antigenic, and the parasites of each cycle contain a completely different glycoprotein. Although the body mounts an antibody response, the parasite can change its surface antigen hundreds of times, always keeping ahead of the host's immune response.

What is the basis for the capacity of these parasites to alter their surface antigens? Investigation of this phenomenon, undoubtedly the most sophisticated mechanism yet devised for evading the immune response of the host, uncovered the first example of "jumping genes" for surface proteins. Each organism has an estimated 300 to 1,000 different genes coding for these variant surface glycoproteins, but only one is expressed at a time. The gene to be expressed is duplicated and then moved to another part of the chromosome where it is expressed. It is not yet known how these parasite genes are selected, how they are turned on and off, or how they are regulated. Nevertheless, studies of the molecular biology of the African trypanosome already have had considerable influence on our understanding of gene rearrangement in general, and promise to uncover mechanisms of gene regulation.

Surface Antigen Repeating Units

Sporozoites, the form of the malaria parasite that matures in the salivary gland of the anopheles mosquito and is injected into the human skin during a bite, are free in the human host's blood for only a few minutes before entering liver cells. Because of their precise specificity for a single antigen, monoclonal antibodies have allowed the detection and characterization of surface proteins on sporozoites. The antibodies are specific to a given species as well as to that stage in the life cycle. The antibodies directed against these surface antigens neutralize sporozoite infectivity, so there has been great interest in both the antibodies and the antigens they have identified.

Genes coding for the sporozoite surface antigens have been introduced into bacteria by recombinant DNA techniques. This allows the production of sufficient antigen to study it in detail. The antigens have been found to have unusual repeating sequences. In the sporozoite of the malaria species *Plasmodium knowlesi*, for example, the unit is made up of a peptide with 12 amino acids repeated 12 times in tandem. The antigen of *Plasmodium falciparum* has a different repeating unit, with 4 amino acids repeated 23 times. It will be interesting to learn how common such repeating antigen units are in other parasites and what evolutionary pressures may have led to their development. In a more practical vein, such small reactive units lend themselves to the production of synthetic peptide antigens that may be used to develop vaccines effective against malaria.

Kinetoplast DNA

Kinetoplast DNA (kDNA) is a form of mitochondrial DNA found in some protozoans such as trypanosomes and leishmania, which is essential for their survival. It has a remarkable structure consisting of thousands of DNA circles interlocked in a network. A minor portion of these circles, the maxicircles, code for mitochondrial proteins. The majority, the minicircles, are only about 1,000 base pairs in size. These do not appear to code for any protein, and their function is not known. Minicircles are the only DNA in nature known to have a region of bent DNA helix. It is of great interest to learn both the function of this unusual DNA conformation and how its nucleotide sequence induces the curvature.

Gene Regulation During Life Cycle Transformations

Parasites undergo profound changes during the various stages of their life cycles, and work on the molecular biologic basis of these changes is in its infancy. The next few years should see dramatic progress in work on the

molecular basis for developmental transformation in many different protozoan and helminthic parasites.

Leishmania, for example, go from a flagellated protozoan form in the insect host to a smaller form without flagella when in a mammalian macrophage. This change is reversed after the protozoa are taken up by a fly when it bites an infected animal. The amount of tubulin, a structural protein of the cytoskeleton, decreases as the parasite goes from the flagellated to the nonflagellated form and then increases when the parasite returns to the flagellated form. In this case of transformation, the control of the genes for tubulin occurs at the level of messenger RNA processing.

IMMUNOLOGY

Studies in immunology have concentrated in several areas. These include mechanisms the host can muster that the parasite cannot escape, mechanisms of immune evasion, and the role of the immune response in causing tissue damage. The information obtained has been useful in developing better diagnostic reagents and forms the basis for the development of protective vaccines.

Protective Mechanisms of the Host

Studies on host responses to parasites have revealed some novel systems. For example, one such system was found while studying the antibody-dependent ability of host cells to kill schistosomes. These studies led to the first demonstration that the eosinophil, a white blood cell, can act as a killer cell. These findings have now been applied to other areas where it has been shown that eosinophils play an important part in producing tissue damage in certain types of heart disease, in inflammation, and in allergic diseases such as in asthma.

As another example, work on the immune response to ticks has highlighted the role of basophils, another type of white blood cell, and mast cells in producing vascular perme-

ability in the early stages of cell-mediated immune reactions. Furthermore, studies of the schistosome-linked granuloma (an inflammatory nodule) were the first to demonstrate suppression of lymphocyte hormone production by T-cells. (T-cells and lymphocyte hormones are important regulatory components of the immune system.)

Mechanisms of Immune Evasion

Parasites have developed many ways of evading the human host's defenses. Some change their surface antigens, some masquerade as the host by taking up host molecules onto their surfaces, and others simply shed their surface antigens. Some have enzymes on their surface that destroy antibodies. Macrophages are the body's major cell for engulfing foreign particles, and they usually kill the organisms they engulf. Some parasites have devised ways of avoiding the effects of the toxic substances present in the lysosomes of the macrophage, although how they do this is not known. Yet other parasites suppress the host's immune attack.

Although at present most of our knowledge is simply descriptive, future studies should elucidate the mechanisms for accomplishing all these forms of evasion. Information about the effects of parasites on the immune system would undoubtedly enhance our understanding of how the immune system functions in many other types of diseases.

Immunopathology

Studies on the mechanisms of tissue damage in schistosomiasis demonstrated that the host reactions to egg antigens are the main factors producing pathology. Further studies on this process were the first to show that mononuclear cells of the host release factors that promote fibrosis. The mechanisms of pathology induced by many different parasites merit further study. Of particular interest are the ways parasites induce autoimmune

reactions such as those postulated in infections caused by *Trypanosoma cruzi*, the mechanism of blindness caused by *Onchocerca volvulus*, the process of sequestration of infected red blood cells leading to malaria damage in the brain, and the underlying basis for destruction of cartilage in mucocutaneous leishmaniasis.

MEMBRANE BIOLOGY AND CELL BIOLOGY

Unusual Features of Parasitic Membranes

The first interactions between the parasite and the host are membrane-membrane interactions. Many parasites have membranes that are different from those in mammalian cells, and considerable effort has been directed at learning about the function and structure of parasite membranes. As a result, several interesting discoveries have emerged.

One example is the finding that the variant surface glycoprotein of African trypanosomes is attached to the membrane by a novel protein-lipid bond. A newly recognized enzyme is involved in destroying this bond. Another unusual finding concerns an enzyme on the surface membrane of *Trypanosoma cruzi*. This organism has a neuraminidase, an enzyme that cleaves off the most important sugar group on the surface of mammalian cells, namely, sialic acid. The role of this enzyme should be studied, especially as it relates to parasite survival and tissue injury. The question of whether similar enzymes are present on other parasites also is of interest.

Parasite-Host Membrane Interactions

Work is being carried out on the mechanism by which malarial merozoites (the mobile infective stage) invade red blood cells. Glycophorin, a glycoprotein on the red blood cell surface, appears to be an important part of the receptor for the parasite. How parasites disrupt the rigid cytoskeleton of the red blood cell so they can enter it is unknown, as is the

source for the special membrane that surrounds the parasite once it is in the red blood cell. Furthermore, parasite antigens soon appear on the outer surface of the red blood cell. To reach the surface, parasite antigens must traverse the parasite's own membrane, then the special membrane surrounding the parasite, and finally the red blood cell membrane. The mechanism for accomplishing this voyage is unknown.

Alteration of the red blood cell surface is important in enabling the red blood cells that contain the parasite of *Plasmodium falciparum*, the most common of the four malaria species, to lodge in the vascular bed of certain organs such as the brain. These surface antigens also undergo antigenic variation by unknown mechanisms. The spleen can alter the expression of these parasite antigens on the red blood cell surface, but how it does so is another mystery to be solved.

Of interest is the recent discovery that *Entamoeba histolytica*, another protozoan parasite, kills host cells by injecting a protein into the host cell membrane that causes an ion flux and subsequent osmotic lysis of the target cell. These findings have stimulated a search to see whether analogous proteins are involved in cytotoxicity induced by T-lymphocytes.

Clearly, studies on these membrane effects will have ramifications affecting much more than our knowledge of the parasites themselves.

BIOLOGY OF INSECT VECTORS

An attack on the insects that transmit particular pathogens remains a major focus for most programs aiming to control parasitic diseases. Although chemical insecticides provide the major tool for this purpose, their effectiveness has steadily diminished because of insect resistance.

New technologies should lead to revised strategies for combating insect vectors. Recent developments in molecular biology, combined

with improved understanding of insect physiology and behavior, should enable us to better understand the mechanisms of insecticide resistance, vector competence—the ability of an insect to transmit a parasite—and the control, feeding behavior, and reproduction of insects. Ultimately, this technology may enable us to genetically modify insect populations in nature, so that the targeted population is less capable of transmitting pathogens or less susceptible to becoming resistant to insecticides.

Such research, both basic and applied, should have far-reaching benefits, affecting not only the transmission of known disease but also important aspects of agriculture.

BIOCHEMISTRY AND PHARMACOLOGY

The very nature of parasitism implies that the parasite has metabolic needs that the host supplies and, therefore, that the parasite must have metabolic pathways that differ from those of the host. Novel metabolic pathways and even new cell organelles are being discovered. These have led to the design of compounds that can inhibit these unique pathways and thus control some parasites and insects without affecting their hosts. A few examples are described below.

Unusual Metabolic Pathways

In general, parasitic protozoans cannot synthesize purines, which are chemical precursors of nucleic acids. They have therefore developed elaborate pathways to utilize purines from the host. Many of these pathways differ from one protozoan to another. Study of these pathways has greatly enhanced our knowledge of purine metabolism in general and may lead to development of drugs that can kill protozoans but are not toxic to mammalian cells. Polyamines also are required for DNA replication and cell differentiation. Trypanosomes and leishmania have

simplified metabolic pathways for biosynthesis of polyamines, which may be susceptible to specific inhibition.

Unusual Organelles

Parasites have been found to contain several novel organelles. One of these, the glycosome, found in trypanosomes, contains the enzymes for glycolysis; its discovery encourages research toward development of specific inhibitors. Another novel organelle is the hydrogenosome found in trichomonas. This organelle contains pyruvate kinase and pyruvate dehydrogenase, two enzymes used in producing the ATP needed by the cell. These hydrogenosomes chemically reduce the drug metronidazole to a toxic compound that kills the parasite. The host cells, which lack this organelle, are spared.

The Neuromuscular Junction

The neuromuscular junction of certain helminths contains receptors for GABA (gamma-aminobutyric acid), which also is a neurotransmitter in the human brain. Ivermectin, a drug that enhances the activity of these receptors, has a profound effect on helminths. It causes paralysis that prevents them from moving or eating. It is exceedingly potent, with less than milligrams per acre of pastureland protecting cattle against helminth parasites. Because this drug cannot traverse the blood-brain barrier, it is nontoxic to humans. Further studies using ivermectin have shown that it also acts on the GABA receptor complex of arthropods. Additional studies with this drug should greatly increase our understanding of insect physiology and yield knowledge of GABA receptors in higher organisms.

Juvenile Hormone

Juvenile hormone (ecdysin) is involved in the differentiation and molting of insects and has recently been found in helminths. Further

study of the regulation of molting and differentiation of helminths and the role of ecdysin in this process should lead to entirely new approaches to control of some of these parasites.

APPLICATION TO DISEASES

Increased understanding of the basic biology of parasitism should provide many opportunities for combating diseases caused by these organisms. This is especially important now that traditional public health measures that had helped to control some of these diseases are no longer sufficient by themselves. An example of the need for new methods can be seen in the case of malaria. After World War II, there was great hope that this disease could be eradicated by using DDT to kill the mosquitoes in houses and chloroquin to treat infections in people. Indeed, malaria was eradicated from the United States and around the Mediterranean and decreased markedly in Asia and South America. In the early 1960s in Sri Lanka, the number of cases per year fell from more than 1 million to 18. However, within 5 years, there were again almost a million cases, due to a combination of DDT and chloroquin resistance and to the difficulty in maintaining this costly control program. Now malaria has reached serious epidemic proportions in parts of Asia and South America and continues unabated in Africa where it was never controlled due to the behavior of the local mosquitoes, which, instead of landing on the sprayed walls of the house after biting, go outside. Malaria resistant to the present drugs has become an alarming problem.

The belief that parasitic diseases disappear with modernization and industrialization is not always correct. For instance, the prevalence of schistosomiasis has increased hand in hand with the development of hydroelectric dams required for energy and irrigation projects necessary for improved agriculture. The large lakes behind these dams have added thousands of miles of waterfront

and have increased the contact between people and the infected snails that transmit this parasite. Another example is the increase in leishmaniasis in the Amazon where people who are expanding towns and building roads come into contact with sandflies that live in the forest and transmit this infection. Applying insecticides to the forests is neither effective nor feasible.

FUTURE RESEARCH OPPORTUNITIES

There is a great need now to broaden the entire base of biologic research on parasites. This is an opportune time because many of the new concepts and biotechnologies make it possible to solve some of the important questions concerning the biology of parasitism. A multidisciplinary approach is required.

Most of the productive research thus far employing the new biotechnologies has been on two parasites, the malaria parasite *P. falciparum* and the African trypanosome of cattle, *T. brucei*. Much less work in molecular biology, for instance, is being done on the African trypanosomes that infect humans, or on other protozoans and the many helminths.

The main reason for the early focus on *P. falciparum* and *T. brucei* is the relative ease, compared with other parasites, with which these two parasites can be cultured and manipulated in the laboratory. There is much excitement over prospects of a vaccine against the infective form of *P. falciparum*, the sporozoite, but little work has been carried out on the other three species of malaria that infect humans. There is also work in progress on the development of vaccines to the other stages of the *P. falciparum* parasite because it is still not clear how effective the vaccine against the sporozoite will be. The sporozoite vaccine has worked in laboratory tests on rodents, but must now be developed for humans. One problem is that the vaccine will have to work completely in the short time that the sporozoite is present in the blood. An effective adjuvant must be identified, one that will allow

for a strong enough response for the vaccine to be clinically effective.

It is now important to take advantage of new technologies to study other parasites. This will require establishing cultures of these organisms in vitro, developing suitable animal models, and establishing appropriate life cycles in the laboratory. Such work is time-consuming, may involve many false starts, and requires cooperation of persons in many disciplines and long-term support.

Molecular biology is providing several new methods for generating antigens needed to form the basis of vaccines. These methods include laboratory synthesis of peptides, producing proteins in microorganisms by recombinant DNA techniques, and incorporation of genes coding for protective parasite antigens into the DNA of attenuated viruses used for unrelated vaccines. The essence of the problem is no longer how to produce antigens by recombinant techniques, but which antigens to produce and how to present them effectively to the immune system in clinically acceptable formulations.

Molecular biologic techniques also are being used for better diagnosis. For instance, the nucleotide sequence of the kinetoplast DNA found in certain protozoans varies among the different species. Using DNA hybridization to identify the kDNA, it is now possible to determine in 24 hours instead of 3 months whether a leishmania infection involves a benign or virulent species. Similar techniques should be applicable to other parasites. Furthermore, the production of species-specific monoclonal antibodies against parasites causing malaria, leishmaniasis, schistosomiasis, and other diseases is leading to the development of greatly improved diagnostic reagents and identification of relevant antigens for the development of vaccines.

The study of the genes that regulate the transformation of the parasite through its different stages should result in novel ways of interrupting the life cycle. Studies of the molecular biology of parasites have already broadened our understanding of molecular

biology in general. For instance, it was through the study of malaria that an enzyme, mung bean nuclease, was found recently to have the unique property of cutting genes out of DNA without cutting in the coding region, so that genes can be studied and cloned much more easily than before.

Basic immunologic studies of parasites should be expanded to cover several other areas beyond development of vaccines and diagnostic reagents. For instance, the study of the mechanisms of immune evasion should lead to new methods of overcoming the parasites, and those methods should also be adaptable to such other types of organisms as bacteria, viruses, and fungi. Work on immunopathology, especially possible autoimmune mechanisms, is required to assure that the antigens proposed for vaccines are not also those that can induce pathology detrimental to the host. This type of study is important, for instance, in *T. cruzi* infections, because it has been postulated that the heart disease resulting from this infection may be caused by an autoimmune process.

Basic research on the novel biochemical mechanisms that parasites have evolved is currently one of the most neglected aspects of parasitology. Work in this area should lead to the development of new drugs that are needed to treat some of these diseases. For instance, the major drug for treating African

trypanosomiasis is Suramin, first synthesized in 1917. It is a very toxic drug and is effective only in the early stages of the disease, before the parasite reaches the brain and produces the symptoms that give the disease its common name, sleeping sickness. There are no drugs to treat *T. cruzi* infection, and those available against the leishmania organisms were first produced decades ago and are quite toxic.

Studies of the biology of insects that carry and transmit parasites should be expanded to include the application of molecular biology. Alternative methods of insect control, including the genetic modification of insect populations in nature, should result. Investigations in this area should not only help control some of these parasite diseases but also should be applicable to problems in agriculture and animal husbandry.

Despite the enormous potential of research on the basic biology of parasites, relatively few scientists are working in this area, and many more should be encouraged to do so. The foregoing examples of present work on the biology of parasitism should make clear the excitement of imminent discovery and the promise that the research not only will reap new scientific knowledge, but also will enable applications of immense practical consequences. Further study offers tremendous opportunity for progress.